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39. (New) The cell of claim 1 wherein the essential gene does not encode a trans regulatory element for the lethal gene.

40. (New) The cell of claim 1 wherein the essential gene does not regulate expression of the lethal gene.

Remarks

Claims 1-32 and 35-40 are pending. Claims 33 and 34 have been canceled. Claims 39 and 40 have been added to more clearly recite what applicants consider to be their invention. Claim 39 requires that the essential gene not encode a trans regulatory element for the lethal gene. Support for new claim 39 appears at least on page 19, especially on lines 3-5, where trans regulatory element is defined as a molecule or complex that modulates the expression of a gene. Claim 40 requires that the essential gene does not regulate expression of the lethal gene. Support for new claim 40 appears at least from page 31, lines 27, to page 32, line 4, where it is clear that regulatory elements in the claimed Environmentally Regulated Viability System are separate from the essential and lethal genes. A copy of all of the pending claims is attached to this Amendment and Response in an appendix.

Applicants note that the Office Action incorrectly indicates that only claims 1-5, 8-14, 16, 20, 23, 24, 27-29, and 37 are pending. In fact, claims 1-32 and 35-40 are pending. Claims 5-7, 15, 17-19, 21-22, 25, 26, 30-36, and 38 had been withdrawn from consideration as being drawn to a non-elected invention (although claims 30-35 are not properly withdrawn; see discussion below). It appears that the claims formerly on appeal were erroneously considered to be

equivalent to the claims pending. In this regard, applicants note that only rejected claims are to be appealed. Correction of the record is respectfully requested.

The present invention is a microbial cell having an Environmentally Limited Viability System (ELVS) such that the cell is viable in a permissive environment and non-viable in a non-permissive environment (see page 5, lines 14-19). The ELVS achieves this environmentally specified viability using two components, an essential gene and a lethal gene (see page 5, lines 16-19; claims 1 and 27). Essential genes and lethal genes are specifically limited in the claims, and defined in the specification (see pages 11-17, especially pages 11 and 16), to refer to mutually exclusive genes having mutually exclusive effects. As recited in the claims, an essential gene is a gene whose expression is essential to the viability of the cell (see page 11, lines 15-16). A lethal gene is a gene whose expression is lethal to the cell (see page 16, lines 7-8). Thus, a lethal gene according to the claims cannot be an essential gene, and an essential gene cannot be a lethal gene. The expression of the lethal gene and the expression of the essential gene, as recited in the claims, are also mutually exclusive. The essential gene is regulated such that the essential gene is expressed in the permissive environment but is not expressed in the non-permissive environment (see page 11, lines 16-19). The lethal gene is regulated such that the lethal gene is expressed in the non-permissive environment but is not expressed in the permissive environment (see page 16, lines 8-10).

Permissive environments at a temperature of about 37°C and non-permissive environments at a temperature of less than about 30°C, as recited in claims 2 and 28, are described on page 20, lines 8-21. Permissive environments inside an animal and non-permissive

environments outside of an animal, as recited in claims 3, 23, and 29, are described on page 20, lines 8-21. Localization of essential genes and lethal genes on a vector, as recited in claim 4, is described on page 12, lines 7-8, and page 17, lines 11-12, respectively. An ELVS vector having two lethal genes and vector pMEG-104, as recited in claims 8 and 9, is described on page 24. Use of a host cell that is a gram-negative enteric bacterium of the genus *Escherichia* or *Salmonella*, as recited in claims 10-12, is described on pages 30-31. Regulation of expression of the essential gene by the expression product of a regulatory gene, as recited in claims 13, 14, and 37, is described on page 19, lines 3-10. An environmentally regulated replication gene required for replication of the ELVS vector, as recited in claim 16, is described from page 17, line 24, to page 18, line 8. Inclusion of an expression gene encoding a desired expression product, as recited in claims 20 and 24, is described on page 35, lines 13-18.

Rejection Under 35 U.S.C. § 112, second paragraph

Claims 1-5, 8-14, 16, 20, 23, 24, 27-29, and 37 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite.

The claims were considered indefinite in the recitation of "permissive environment" and "non-permissive environment" on the basis that these terms are allegedly relative and lack a clear definition in the specification. Applicants initially note that the terms "permissive" and "non-permissive" are not relative. A "permissive" environment is defined in the specification (page 11, lines 1-4) as "an environment in which microorganisms incorporating an Environmentally Limited Viability System are viable." A "non-permissive" environment is defined in the specification (page 11, lines 5-7) as "an environment in which microorganisms incorporating an

Environmentally Limited Viability System are non-viable." Thus, these terms are not only absolute but are also clearly defined in the specification. The fact that they are defined in reference to another condition (cell viability) does not affect the definiteness of the terms.

Rejections Under 35 U.S.C. § 102

Claims 1-5, 8, 10-14, 16, 20, 23, 24, 27-29, and 37 were rejected under 35 U.S.C. 102(e) as being anticipated by U.S. Patent No. 5,681,745 to Szafranski et al. Applicants respectfully traverse this rejection.

Szafranski et al. discloses a cell containment system involving a lethal gene (for example, a gene encoding a biotin-binding protein) regulated by an environmental condition. Szafranski et al. also discloses that the cell containment system can include a gene that regulates the expression of the lethal gene. Szafranski et al. fails to disclose or suggest the use of an environmentally regulated essential gene that is expressed under conditions where the lethal gene is not, and not expressed under conditions where the lethal gene is expressed.

The claimed cells require both an environmentally regulated lethal gene **and** an environmentally regulated essential gene. Since Szafranski et al. fails to disclose or suggest such a regulated essential gene, Szafranski et al. fails to disclose each and every feature of the claimed cells. Accordingly, Szafranski et al. fails to anticipate the claimed cells and method.

Claims 1-5, 8-14, 16, 20, 23, 24, 27-29, and 37 were rejected under 35 U.S.C. 102(e) as being anticipated by U.S. Patent No. 5,702,916 to Molin et al. Applicants respectfully traverse this rejection.

Molin et al. discloses a cell containment system involving a lethal gene (for example, the *hok* gene) regulated by an environmental condition. Molin et al. also discloses that the cell containment system can include a gene that regulates the expression of the lethal gene (for example, the *sok* gene). Molin et al. fails to disclose or suggest the use of an environmentally regulated essential gene that is expressed under conditions where the lethal gene is not, and not expressed under conditions where the lethal gene is expressed.

The claimed cells require both an environmentally regulated lethal gene **and** an environmentally regulated essential gene. Since Molin et al. fails to disclose or suggest such a regulated essential gene, Molin et al. fails to disclose each and every feature of the claimed cells. Accordingly, Molin et al. fails to anticipate the claimed cells and method.

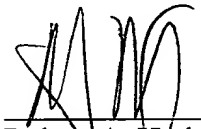
Restriction Requirement

In the Office Action mailed December 23, 1997, claims 30-35 were indicated as withdrawn from consideration as being drawn to a non-elected invention. However, there is no basis in the prosecution history for holding claims 30-35 withdrawn, and applicants respectfully request examination of these validly pending and elected claims. In the Office Action mailed October 18, 1996, a restriction requirement was set forth where the claims were divided into two groups; Group I, claims 1-29, drawn to an isolated microbial cell, and Group II, claims 30-35, drawn to a method of vaccination. The Office Action noted (page 3, lines 5-9) that if "claim 30 were amended to require both an essential gene and a lethal gene in accordance with claim 1, then the restriction between these two inventions would be withdrawn." In response (see Response mailed November 18, 1996, paragraph bridging pages 4 and 5), applicants amended

claim 30 just as suggested to require both an essential gene and a lethal gene. In the next Office Action, mailed April 15, 1997, this amendment was duly noted and the restriction requirement between the two groups of claims was withdrawn (see page 2, lines 15-17). In the Office Action mailed December 23, 1997, and in the latest Office Action, mailed November 13, 1998, claims 30-35 were not rejected. Since no basis exists, either on the record or in fact, to withdraw claims 30-35 from examination, applicants respectfully request an indication of allowability of claims 30-32 and 35 or some other action consistent with examination (claims 33 and 34 are now canceled).

Allowance of claims 1-32 and 35-40 is respectfully solicited.

Respectfully submitted,



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Teresa R. Spratt

Date: March 15, 1999

Appendix: Claims As Pending After Amendment

1. An isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable in a non-permissive environment, the system comprising

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment; and

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment.

2. The cell of claim 1 wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

3. The cell of claim 1 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal.

4. The cell of claim 1 wherein the essential gene, the lethal gene, or both, is carried on an extrachromosomal vector.

5. The cell of claim 4 wherein the lethal gene is carried on an extrachromosomal vector and expression of the lethal gene is regulated by an expression product of a regulatory gene.

6. The cell of claim 5 wherein the expression product of the regulatory gene inhibits expression of the lethal gene and is expressed or active only in the permissive environment.

7. The cell of claim 5 wherein the expression product of the regulatory gene induces expression of the lethal gene and is expressed or active only in the non-permissive environment.

8. The cell of claim 4 wherein the vector has two lethal genes.

9. The cell of claim 8 wherein the vector comprises pMEG-104.

10. The cell of claim 1 wherein the cell is a gram-negative bacterium.

11. The cell of claim 10 wherein the gram-negative bacterium is an enteric bacterium.

12. The cell of claim 11 wherein the genus of the enteric bacterium is selected from the group consisting of *Escherichia* and *Salmonella*.

13. The cell of claim 1 wherein expression of the essential gene is regulated by an expression product of a regulatory gene.

14. The cell of claim 13 wherein the expression product of the regulatory gene inhibits expression of the essential gene and is expressed or active only in the non-permissive environment.

15. The cell of claim 13 wherein the expression product of the regulatory gene induces expression of the essential gene and is expressed or active only in the permissive environment.

16. The cell of claim 4 wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed in the permissive environment and is not expressed in the non-permissive environment.

17. The cell of claim 16 wherein expression of the replication gene is regulated by an expression product of a regulatory gene.

18. The cell of claim 17 wherein the expression product of the regulatory gene inhibits expression of the replication gene and is expressed or active only in the non-permissive environment.

19. The cell of claim 17 wherein the expression product of the regulatory gene induces expression of the replication gene and is expressed or active only in the permissive environment.

20. The cell of claim 1 further comprising an expression gene wherein the expression gene encodes a desired expression product.

21. The cell of claim 20 wherein the desired expression product is an antigen.

22. The cell of claim 21 wherein the antigen is selected from the group consisting of bacterial antigens, viral antigens, plant antigens, fungal antigens, insect antigens, and non-insect animal antigens.

23. The cell of claim 1 for use as a vaccine, wherein the cell is viable when in the an animal and non-viable when outside of the animal, the essential gene is expressed when the cell

is in the animal and is not expressed when the cell is outside of the animal, and the lethal gene is expressed when the cell is outside of the animal and is not expressed when the cell is in the animal, wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

24. The cell of claim 23 further comprising an expression gene wherein the expression gene encodes a desired expression product.

25. The cell of claim 24 wherein the desired expression product is an antigen.

26. The cell of claim 25 wherein the antigen is selected from the group consisting of bacterial antigens, viral antigens, plant antigens, fungal antigens, insect antigens, and non-insect animal antigens.

27. A method of making a cell strain with environmentally limited viability comprising stably introducing into a cell

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment;

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment,

wherein the cell strain is viable in a permissive environment and non-viable in a non-permissive environment.

28. The method of claim 27 wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

29. The method of claim 27 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal.

30. A method of inducing immunoprotection in a warm-blooded animal comprising

administering to the animal a vaccine comprising a microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable when in the animal and non-viable when outside of the animal, the system comprising

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal; and

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is outside of the animal but not when the cell is in the animal.

31. The method of claim 30 wherein the system further comprising an expression gene wherein the expression gene encodes an antigen.

32. The method of claim 31 wherein the antigen is selected from the group consisting of bacterial antigens, viral antigens, plant antigens, fungal antigens, insect antigens, and non-insect animal antigens.

35. The method of claim 30 wherein the essential gene, the lethal gene, or both, is carried on an extrachromosomal vector, and wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal.

36. The cell of claim 5 wherein the absence of a functional expression product of the regulatory gene derepresses expression of the lethal gene and wherein the expression product is not expressed or is inactive only in the non-permissive environment.

37. The cell of claim 13 wherein the absence of a functional expression product of the regulatory gene derepresses expression of the essential gene and wherein the expression product is not expressed or is inactive only in the permissive environment.

38. The cell of claim 17 wherein the absence of a functional expression product of the regulatory gene derepresses expression of the replication gene and wherein the expression product is not expressed or is inactive only in the permissive environment.

39. The cell of claim 1 wherein the essential gene does not encode a trans regulatory element for the lethal gene.

40. The cell of claim 1 wherein the essential gene does not regulate expression of the lethal gene.